Fluorescent labeling and visualization of human induced pluripotent stem cells using TAL effector nucleases

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Abstract

Background

The visualization of induced pluripotent stem (iPS) cells using fluorescent techniques is useful for the in vivo evaluation of iPS-derived functional cells following differentiation and distribution of the transplanted cells. The data obtained using the fluorescently labeled iPS cells would lead to amelioration and validation of protocols directing the differentiation of iPS cells into functional cells. In this study, we established enhanced green fluorescent protein (EGFP)-labeled iPS cells to enable their easy visualization.

Methods

Human iPS cells were transfected with (a) two transcription activator-like effector nuclease (TALEN) vectors targeted to the adeno-associated virus integration site 1 (AAVS1) locus and (b) the targeting vector carrying the homology arms, EGFP gene, and a drug-selection marker.

Results

Several puromycin-resistant clones were obtained after transfection of the targeting vector and corresponding TALEN-expressing vectors. EGFP expression in these clones was observed using a fluorescent microscope. Clones were examined for specific recombination, which revealed precise targeting at the AAVS1 locus. Furthermore, EGFP protein expression was sustained after directed differentiation into a hepatic lineage.

Discussion

We were successful in evaluating the behavior of iPS-derived hepatic cells. The data suggest that genomic knock-in at the AAVS1 locus is suitable for long-term observation of iPS-derived cells. Manipulation of the iPS genome can also be applied for the purification of hepatic cells during iPS cell differentiation by introducing the fluorescent protein under the regulation of a hepatic cell-specific promoter. Another application involves gene correction of iPS cells from patients with hepatic disease for regenerative medicine.

Introduction

Since the development of induced pluripotent stem (iPS) cell technology, the generation of functional cells from pluripotent stem cells has been eagerly anticipated. Liver transplantation is considered a promising option for patients with serious hepatic disease; however, there is a serious shortage of donor livers available for transplantation, which continues to worsen. Hepatocytes generated by the differentiation of human iPS cells can be used as a cellular source for transplantation, for drug screening, and in in vitro toxicology studies¹. To this end, several investigators aimed to develop differentiation protocols directing the differentiation of iPS cells into hepatocytes; the derived hepatocytes, however, were of lower efficiency and quality than freshly isolated adult hepatocytes. Fluorescent labeling of iPS cells is useful for evaluating iPS cell differentiation and distribution after transplantation into model animals. The data obtained from such studies will facilitate the amelioration and validation of protocols directing the differentiation of iPS cells into hepatocytes. In this study, we established enhanced green fluorescent protein (EGFP)-labeled iPS cells to enable their visualization using fluorescence microscopy. The drawback of using conventional procedures, such as virus-mediated gene transfer, for introducing the EGFP gene is downregulation of the fluorescent marker after induction of differentiation. On the other hand, the efficiency of conventional homologous recombination is quite low in the human pluripotent stem cells.

In this study, we established EGFP-labeled iPS cells using a transcription activator-like (TAL) effector nuclease (TALEN)-mediated knock-in approach to facilitate the visualization of iPS cell-derived hepatic cells².

Methods

Human iPS cells were maintained using a standard procedure. These cells were transfected with (a) two TALEN vectors targeted to the AAVS1 locus and (b) a targeting vector carrying the homology arms and the EGFP gene along with a drug selection marker. Following transfection, the cells were cultured in medium containing puromycin. The expression of the fluorescent protein was examined in drug-resistant clones using a fluorescent microscope. Clones

were also examined for precise recombination at the AAVS1 locus. Hepatic differentiation was performed using a standard procedure^{3,4}.

Results

We employed TALEN technology to introduce a fluorescent protein, EGFP, into the AAVS1 locus of the human iPS cells. The AAVS1 locus is known to have open chromatin configuration in various organs in humans, similar to the ROSA26 locus in mice. Following transfection of the targeting vector and corresponding TALEN-expressing vectors, several drug-resistant clones were obtained. These clones also expressed EGFP protein, as observed using the fluorescent microscope. Precise recombination at the AAVS1 locus was confirmed in these clones. To evaluate whether EGFP expression could be sustained during directed differentiation of iPS cells into a hepatic lineage, we induced differentiation in the AAVS1 knock-in iPS cells. Sustained EGFP expression was observed in in vitro 2D culture, at least at the stage when mature hepatocyte-like cells were developed (Fig. 1). Gene expression was analyzed by qPCR (Fig. 2A, B)⁵. Furthermore, albumin protein secretion was detectable by ELISA (Fig. 2C).

Discussion

Compared with the TALEN method, other available genome editing technology such as the zinc-finger nuclease method suffers from the limitation of not having a select target sequence, because each zinc-finger domain recognizes three nucleotides⁶. CRSPR/Cas is another emerging technology that seems very promising; however, the specificity of this technique is not well established currently⁷. Hence, we employed the TALEN technology in this study. A sustained EGFP protein expression was obtained in the AAVS1 knock-in iPS-derived hepatic cells. Thus, we succeeded in visualizing human iPS cells throughout the hepatic differentiation process, from the undifferentiated state to mature hepatocyte-like cells. Such a manipulation of the iPS genome can also be applied for the purification of hepatic cells during iPS cell differentiation by introducing the fluorescent protein under the regulation of a hepatic cell-specific promoter. An additional application involves gene correction of iPS cells from patients with hepatic disease for regenerative medicine.

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Figure Legends

Figure 1

Sustained EGFP expression during directed differentiation of iPS cells into a hepatic lineage. Sustained EGFP expression is observed at least at the stage of mature hepatocyte-like cells in in vitro 2D culture.

Figure 2

A, B) Gene expression analysis by qPCR and C) ELISA for examining albumin protein secretion.

Figure.1 Sekine, et. al.

Figure.2 Sekine, et. al.